The Chromosome Cycle of Gregarines, with Special Reference to Diplocystis schneideri Kunstler.

By

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With Plates 12 to 15.

Contents.

INTRODUCTION.

ALTHOUGH numerous papers have been published on the life-histories of Gregarines, no one has, up to the present, attempted to study in detail all nuclear phases in a single life-cycle. As a rule the earliest and the latest stages have received but scanty attention, while the intermediate ones have been unduly stressed in consequence. Our knowledge of this group is thus, in this respect, lop-sided and scrappy. On certain points a considerable body of facts is available, but the lack of any connected thread has prevented their real value from being appreciated; for, in order that the different stages in a life-history may be properly understood, it is obvious that all the stages must be known.

The study of *Diplocystis schneideri* was undertaken to try to supply the missing thread in at least one gregarine life-cycle. My intention was to study every nuclear phase with care. This particular parasite was selected on account of its large size, and also because a constant supply of material could be assured. It was intended to supplement the work done on *Diplocystis* by the investigation of other Gregarines: but unfortunately my work was interrupted by the war, and had to lie untouched for over four years. When

at last I could resume my researches the time at my disposal was very limited. I therefore confined myself to finishing my work on Diplocystis, leaving the other Gregarines for some future occasion.

In this paper I shall concern myself chiefly with the nucleus and the various nuclear changes. The general morphology of Diplocystis is not of particular interest or importance, and I shall touch that side of the subject only briefly.

My work on Diplocystis schneideri has been carried out, to a great extent, in association with Mr. Clifford Dobell, to whose researches—still, for the most part, unpublished—on the coccidian Aggregata eberthi it forms a counterpart. In an earlier joint paper (Dobell and Jameson, 1915) we have already recorded the chief results of our work, in so far as they concern the behaviour of the chromosomes throughout the life-histories of these two Sporozoa. It will be convenient to state very briefly here what these results were. In the first place, we found that our observations were in complete agreement in showing that the nuclear divisions, at all stages in the life-cycles of the organisms studied, are mitotic. Amitosis, resolution into chromidia, disappearance of the nuclei at certain stages—all of which have been described in the Coccidia and Gregarines—do not occur during normal development. Secondly, we found that the number of chromosomes formed during mitosis is constant. The chromosomes are neither indefinite nor variable in number, but as definite and constant as in any of the multicellular animals and plants. Thirdly, we found that both the organisms studied showed, with the greatest clearness, that the chromosome cycle is remarkable, differing considerably from that which, on analogy with the Metazoa, previous workers had been led to expect. We found that the nuclei of Aggregata and Diplocystis contain, at every stage in the life-history save one, a chromosome complex which consists of a single set of differentiated elements, constituting a haploid—not a diploid—group. The one exception is the zygote nucleus,

which is formed, as in other organisms, by the fusion of two gamete nuclei, each containing a single (haploid) set of chromosomes. The zygote nucleus is the only diploid nucleus in the life-cycle. In agreement with these findings, we found further that reduction, or meiosis—the halving of the chromosome number—occurs immediately after, and not before, fertilization. The first division of the zygote nucleus is a true reduction division, comparable with the reduction divisions which occur in the gametogenesis of the Metazoa.

It is thus clear that the chromosome cycle in Aggregata and Diplocystis—and, in all probability, in other Coccidia and Gregarines—is radically different from that which has been demonstrated in the Metazoa.

In the present paper I shall describe my own observations upon Diplocystis schneideri as briefly and objectively as possible, without reference to the work of others on other Gregarines, and laying particular emphasis upon the behaviour of the nuclei and chromosomes throughout the life-cycle. In the second part I shall then consider the observations of other workers, in so far as they are relevant, and attempt to show how they should be interpreted. All the discrepant facts can, I think, be reconciled in the light of my own findings; and they already tend to show, I believe, that the conclusions drawn from the study of Diplocystis will probably be found to hold good for the whole group of Gregarines in general.

I wish to express my gratitude to my friend, Mr. Clifford Dobell, F.R.S., for the assistance which he has rendered me at every stage of my investigation. Not only did he suggest to me the line of work which I have attempted to follow, but his advice and criticisms have been invaluable to me during its progress. Indeed, if it had not been for his kindly help and sympathy the work might never have been completed.

The work was commenced at the Imperial College of Science while I held the Fullerton Scholarship from Aberdeen University. Its completion, at the same place, has been made

possible by a grant from the Committee for Scientific and Industrial Research, to which body I wish to express my thanks.

Part I.—The Life-cycle of Diplocystis schneideri, with Special Reference to the Behaviour of the Nuclei and Chromosomes.

A. Material, Methods, and Infection Experiments.

Diplocystis schneideri was originally described by Kunstler in 1887 from the body-cavity of the ship cockroach (Periplaneta americana). The material on which I have worked was obtained from the same insect host, but I have also found a certain number of the bakehouse cockroach (Stylopyga orientalis) to be infected with the parasite. I was fortunate enough to get an almost unlimited supply of P. americana from the Reptile House in the gardens of the Zoological Society. Of these cockroaches 81 per cent. were found to be infected with D. schneideri.

As is well known, it is not easy to fix gregarine material satisfactorily. In common with most workers I have found that good fixation is obtained only with picric acid fixatives. Picro-acetic, in both aqueous and alcoholic solution, was perhaps the most successful; but the fluid of Bouin, and Duboscq's alcoholic modification of it, were extremely good.

In embedding, the objects were not passed directly from xylol into paraffin, but were passed through slightly warmed baths of chloroform and paraffin and through several changes of paraffin in the bath. The food-granules in the protoplasm were found to cut more easily after this graduated embedding process.

As a rule the parasites were stained very lightly with borax carmine before embedding. They were then inspected with a pocket lens. In this way it was possible to sort the specimens into lots roughly corresponding to the stages in the life-history. For example, those in which the nucleus had not

commenced to divide had a prominent nucleus ; those in which the early divisions were taking place had no visible nucleus : those in which the nuclei were collecting round the periphery for gamete formation had a delicate pink zone round the periphery ; those which were flaccid and watery had formed spores. By sectioning a few specimens together from one of the lots, and not sectioning individuals at random, the very laborious work of cutting and searching through many sections for the different stages in the life-cycle was somewhat lessened.

In staining, Dobell's alcoholic iron-alum hæmatein stain was found to be invaluable. In crispness and delicacy of colouring this stain far surpasses any other. Its more general use would, I believe, reveal much that up to the present has been hidden. Heidenhain's iron-alum hæmatoxylin was used to some extent, but did not reveal the finer details. Safranin was also occasionally employed, as was borax carmine for whole mounts.

The spores are extremely difficult to stain. In smears they were only imperfectly stained with acid carmine. No other stain penetrated the spore-wall at all. In thin sections, of course, it was possible to get quite excellent results.

In order to study the dehiscence of the spores I treated them on the slide with mid-gut extract, but this had little or no effect. At times the sporozoites within the spore seemed to move, but the spores never showed any signs of dehiscing. Sixty-five cockroaches were fed on spores. Of these, thirty were examined for dehiscence of the spore by smearing the mid-gut contents on coverslips. In only two of these were the spores found to have opened. The remaining thirty-five were killed at different intervals and the mid-guts were sectioned to find the penetration of the gut by the sporozoites, but in only three cases were young parasites found in the wall.

These experiments are of little value for two reasons. In the first place, since 81 per cent. of the cockroaches used were found to be naturally infected with the parasite, it was impossible to be sure that the young parasites I found were

derived from the spores on which I had fed the cockroaches and not from a previous wild infection. In the second place, it is not possible to keep such active animals as cockroaches in captivity under perfectly normal conditions. They eat little, and probably their digestive juices are not sufficiently normal to cause the spores to dehisce.

B. Macroscopic Appearance and Sketch of the Life-history.

The parasites lie freely in the body-cavity of their host. Almost invariably two individuals are found associated together, although occasionally single parasites or associations of three parasites are found. The former can never complete their life-cycle, and of the latter I have found so few specimens that I have been unable to come to any conclusion regarding their development. In the body of a single cockroach there are to be found parasites at several different stages of development, but the vegetative forms are the most common. These show very clearly the two members of the association (Pl. 12, fig. 1). The younger and smaller pairs resemble two tiny seed-pearls stuck together. Each parasite is rounded in outline, being only slightly flattened at the point of contact with its fellow. They are chalky white in colour. The smallest forms that are found in the body-cavity are easily visible to the naked eye. As growth proceeds the point of contact between the two members of a pair becomes more flattened and larger, but the dual origin of the association is always clearly seen during the vegetative life. The parasites grow to a comparatively large size, a length of 2 mm. or even a little more for the long axis of the pair being not uncommon.

Gradually the pairs lose their original appearance of two little pearls pressed together, becoming oval, but slightly constricted in the middle. Finally the constriction disappears and they become oblong-oval in shape. At the same time a clear zone appears round the periphery of each parasite—a sign that the reproductive period has been entered upon.

The clear zone next disappears and the association loses its pure white colour and becomes watery white. While this change has been going on the "cyst"—for so one can now speak of it—becomes flaccid, the contents becoming very fluid, whereas in the vegetative forms the parasites have very firm bodies.

These stages, as will be seen later on, are associated with spore-formation, the flaccid, watery cyst being filled with spores. Unless these spores are eaten by a cockroach no further development takes place, and the cysts gradually dry and shrivel up, finally becoming withered-looking little brown bodies. If, on the other hand, the host should die or be killed when the spores are ripe, it will almost invariably be eaten by some of its fellows. The spores then dehisce in the gut of the new host, and the liberated sporozoites penetrate the gut-wall, where they associate, evidently quite fortuitously, in pairs. They grow in size, lying immediately under the basal layer of the gut-wall, until at last this ruptures and they fall into the surrounding body-spaces.

Although the parasites may be present in such numbers that every available space inside the body-walls is literally packed tight with them, the host appears to suffer no inconvenience. In particular I examined the reproductive organs to determine whether they had been affected in any way, but even in the most heavily infected cockroaches no sterility seemed to have been produced. In certain of the guts examined, in which numerous parasites were found lying in the walls, the basal membrane showed a very considerable thickening.

c. The Spore and the Sporozoites.

I propose to commence my detailed account of the life-history of Diplocystis schneideri at the point where a cockroach becomes infected with this parasite. Infection is accomplished by a cockroach eating the spores, which, from the nature of the parasite, must be contained within the body

of another cockroach. As I have already pointed out, the spores of D. schneideri are formed within the body-cavity spaces of the cockroach when the parasite has completed its life-cycle. There is, of course, no exit for the spores from these spaces; but as cockroaches are cannibals, and practically always gnaw the abdomen of dead or dying comrades and eat out the abdominal contents, they will almost certainly be eaten by another cockroach. When a high percentage of cockroaches is infected, some of those that are ready to serve for a meal for the others are almost certain to contain ripe spores of Diplocystis.

The spores are 7 to 7·5 μ in length, and oval in shape (Pl. 12, fig. 2). The spore wall is thick, but there is a well-marked, Y-shaped line of dehiscence (Pl. 12, fig. 3), so that when the wall ruptures along this line there is formed a triangular opening through which the sporozoites escape (Pl. 12, fig. 4). Within the spore are eight sporozoites, lying coiled up in such a way that only four can be clearly seen at once. As the spores stain very badly it is difficult to make out the finer details of their arrangement within the wall.

Dehiscence takes place in the mid-gut. It takes thirty hours for ingested materials to pass through the crop and gizzard of the cockroach and down to the end of the mid-gut,[1] and thus the spores are subjected to a prolonged soaking process in the crop before the action of the digestive juices of the mid-gut finally causes them to open.

[1] In order to determine how long it took for food to reach the mid-gut, a number of cockroaches were heavily fed on carmine mixed to a paste with sweetened water. The cockroaches were killed at different intervals of time and their guts were examined. The carmine showed up very distinctly through the thin gut-wall, and one could thus find out easily how far down the gut the carmine meal had got in the given time. I found thirty hours was the time taken, in all cockroaches examined, for food to pass from the mouth to the hind end of the mid-gut. Of course, some of the meal was longer in this part of the gut, as it seems to pass rather slowly through the gizzard, but by thirty hours an appreciable quantity of the meal had passed through.

The sporozoites are some 8 μ in length (Pl. 12, fig. 5). They are roughly spindle-shaped, but tapering more towards one end than the other. Kunstler (1887) describes them as being "club-shaped," thus emphasizing the fact that one end is somewhat thickened. They have a rounded, rather plump appearance. The nucleus, which is situated close to the thicker end, is very small—about 1 μ long—and looks like a tiny vacuole slightly drawn out in the long axis of the sporozoite. At either end of the nucleus there is a small granule of chromatin, and the whole structure thus has the appearance of two tiny chromatin caps separated by a little clear vacuole. The sporozoites advance by a writhing movement (Pl. 12, fig. 6), with the more slender end anterior, the nucleus marking the posterior. I was unable to see any sign of the amœboid process of which Kunstler speaks.

D. Penetration of the Gut-wall and Growth of the Parasite therein.

The sporozoites penetrate the gut-wall in the posterior part of the mid-gut, just in front of the origin of the Malpighian tubes. I have never found any parasites in the anterior half of the mid-gut wall. They penetrate between the long columnar cells of the epithelium, and rapidly make their way through this layer until they come to the basal ends of the cells, where the nuclei are situated, immediately under the basal membrane (Pl. 12, fig. 7). The parasites remain here, and commence their growth.

For a short time the spindle shape of the sporozoite is retained, the posterior end becoming stouter, but without much growth in length taking place (Pl. 12, fig. 8). The posterior end increases most rapidly and becomes at first flattened and then more rounded (Pl. 12, figs. 9 and 10), a few specimens at this stage showing rather an irregular outline, as if the organisms were somewhat amœboid (Pl. 12, fig. 9). While the posterior portion is increasing greatly in size the anterior portion may remain more or less pointed,

so that the young parasite becomes lanceolate in shape (Pl. 12, figs. 13 and 14). Soon, however, all trace of the original spindle form is lost and the parasite becomes oval or roundish (Pl. 12, figs. 10, 16–19). While growth in size is proceeding the cytoplasm becomes very much vacuolated. The larger the parasite the more it is vacuolated (Pl. 12, figs. 12, 14, 16), although the sporozoites and the very young stages in the gut-wall show hardly any vacuolation of their cytoplasm.

The behaviour of the nucleus during this period is of the greatest interest. As soon as the parasite commences to grow, the anterior chromatin particle in the nucleus also increases in size. It forms a round body which stains much less deeply with chromatin stains than the posterior particle (Pl. 12, fig. 11). By its rapid growth the space separating the anterior from the posterior nuclear cap is abolished, so that the posterior cap, which does not at first grow appreciably in size, comes to lie in close contact with the now very large and palely staining anterior cap, which may be called the nucleolus. Although the nucleus is almost completely filled by the large nucleolus, careful inspection always shows that there is a small clear area limited by a delicate membrane surrounding part of the nucleolus (Pl. 12, fig. 12). The nucleus is now many times larger than the sporozoite nucleus.

At first the darkly-staining posterior chromatin granule lies closely pressed against the nucleolus. Soon, however, it is seen that it is actually making its way inside the nucleolus. In the first stages of this process the darkly-staining portion, which I propose to call the micronucleus for reasons which will be discussed later, grows somewhat in size and sends a rounded projection into the nucleolus, so that the micronucleus becomes hour-glass-shaped (Pl. 12, fig. 13). That portion of the micronucleus which projects into the nucleolus then moves still further inwards (Pl. 12, fig. 14), though it seems to remain connected with the outside portion by a delicate strand or tube. Finally, the external portion also moves gradually inside the nucleolus (Pl. 12, fig. 15), so that the

entire micronucleus ultimately lies within it as a round, darkly-staining body (Pl. 12, fig. 16). While this process has been taking place, the nucleolus has become slightly vacuolated (Pl. 12, figs. 14–16).

At first the micronucleus seems to be a homogeneous body, but soon vacuoles begin to appear in it (Pl. 12, fig. 17). It grows in size and becomes slightly paler in staining reactions owing to the presence of vacuoles, but it always stains much more deeply than the surrounding nucleolus (Pl. 12, figs. 17–19). The small clear area already noted as occurring in the early nucleus grows in size very rapidly, and soon surrounds the nucleolus with a large nucleoplasm area bounded by a very delicate membrane (Pl. 12, figs. 14–16). In fixed and stained specimens a delicate network can be made out in it, but I believe this is due to fixation.

The relatively large nucleus thus formed therefore contains within it a large nucleolus inside which is a micronucleus. I propose to call this structure of dual origin the karyosome.

The nucleus does not long remain in this simple form. The nucleolus becomes increasingly vacuolated and irregular in shape, blunt projections which are ultimately "budded" off being formed round the periphery (Pl. 12, fig. 20). The micronucleus also becomes much more vacuolated and irregular in shape (Pl. 12, fig. 21). At times the micronucleus seems to bud off chromatin masses (Pl. 12, fig. 22), but I have not often observed this, and I am uncertain whether it is a normal process or not. Finally the karyosome breaks up and gives rise to a large, much vacuolated portion evidently derived from the micronucleus, as it stains in much the same way, and several less vacuolated, more palely-staining fragments (Pl. 12, fig. 23). These last disappear and the large micronuclear portion breaks up into smaller particles (Pl. 12, fig. 24), which in turn give rise to still smaller fragments chiefly by the growth of vacuoles in them and the budding off of vesicles (Pl. 12, fig. 25). In this way a large number of vacuolated particles are formed in the nucleus, which has all the time been increasing rapidly in size. These particles

are of all sizes and shapes, but one or two fairly large and regular pieces are always present. These larger portions seem to be the reserve from which smaller fragments are constantly being formed. There is thus a continual breaking down of chromatin material going on within the nucleus.

At the commencement of the fragmentation of the karyosome the cytoplasm is much vacuolated and contains no reserve food-granules. It stains very palely. After the process of chromatin disintegration has been going on for some little time, the cytoplasm round the nucleus begins to stain more deeply in a patchy fashion. Chiefly within the more darkly staining areas of cytoplasm, but also to a slight extent all round the nucleus, small spherical bodies begin to appear (Pl. 12, fig. 26). These are reserve food-granules. They increase rapidly in number and size, until the whole parasite becomes packed full of them, so that the cytoplasm almost disappears from view, remaining only as an extraordinarily delicate network between the food-granules.

While these nuclear changes have been going on the parasites generally associate in pairs. This usually takes place before the formation of food-granules, but there does not seem to be any very fixed time for it. Apparently any two parasites may associate, and there are no visible differences between the members of a pair. At first they lie together, touching only at a small point (Pl. 12, fig. 10), but as the organisms grow larger the surface at the point of contact becomes flattened and correspondingly increased in size. When first they unite, sections show the walls at the edges of the area of contact pressed closely together, and in the middle region fused (Pl. 13, fig. 30). Later the fusion becomes more complete, and one sees a slight thickening all round the circumference of the area of contact and fusion (Pl. 13, fig. 31), while the centre of this area is very thin. There is no thick cyst-wall formed round the two parasites, but the thickening at the circumference of the area of fusion and the presence of a delicate membrane round the two associates may correspond to the cyst-wall of other gregarines.

It may be noted here that not infrequently solitary forms of Diplocystis schneideri are met with. These are frequently quite large, but they never complete their development. They are usually found in cockroaches which are not heavily infected with parasites. Occasionally, too, the young parasites make their way inside older ones, but they never advance beyond a very early stage in development (Pl. 13, fig. 29).

The parasite has now completed its growth in the gut-wall, and as it increases in size it bulges into the body-cavity (Pl. 13, fig. 27). Finally the basal membrane ruptures and the parasites become free in the body-spaces of the host. It may happen that as the parasite grows in size it bulges more into the gut than into the body-cavity (Pl. 13, fig. 28), and it is thus possible that some at least may fall into the gut, and, passing to the outside, fail to complete their development.

E. Growth in the Body-cavity.

It will be well at this point to describe a typical Diplocystis pair. The two associates are approximately equal in size (Pl. 12, fig. 1). They are rounded in outline with the surface of contact between the two flattened. Each parasite is surrounded by a delicate membrane (Pl. 13, fig. 31) outside which is a well-marked wall. This wall, which is practically absent in the area of fusion between the parasites, is alveolar in structure (Pl. 13, fig. 31). To the outside of this again there is another very delicate membrane surrounding the two associates and not running into the partition between them. In a section one can see very clearly the thickening which fills the triangular space between the wall of each parasite and the outer membrane at the circumference of the area of contact. Each parasite is filled with spherical food-granules. These stain with hæmatoxylin; they are coloured brown in iodine solution, turning purple on the addition of sulphuric acid; they are soluble in strong, but insoluble in dilute mineral acids, in acetic acid and in alcohol. Judging by these reactions the

granules can be called paraglycogen, but that does not really throw much light on what they are. It is very difficult to see the cytoplasm among the crowd of reserve food-stuff.

The nuclei are relatively large and much alike in the two associates. The nuclear membrane is delicate, and chromatin is present in vacuolated "karyosomes" and small particles (Pl. 13, figs. 32 and 33). All through the nucleus is an irregular network—perhaps caused by fixation—and on this network there is a fine dust of chromatin. The appearance of Pl. 13, figs. 32 and 33, is very characteristic. The larger chromatin particles occupy roughly the centre of a circular area in which the irregular linin network can be seen, while round the periphery of the circle are the small chromatin particles, and outside this again is the network sprinkled with the chromatin dust. These appearances suggest that the larger pieces are giving off smaller portions, which in turn break down into the finest particles. The number of large "karyosomes" is very variable. Sometimes there is only one, sometimes there are several, but I have never seen them absent altogether.

The parasites grow in the body-cavity. How long they remain there before proceeding to gamete formation I am quite unable to say. They pass gradually from the vegetative to the reproductive phase so that it is impossible to draw any line between the two stages.

f. The First Nuclear Division of the Adult Parasite (Gamont).

The first indication of nuclear division is a decrease in the number of small, irregular chromatin fragments in the nucleus. This is accompanied by an increase in the amount of fine chromatin dust (Pl. 13, fig. 34). At the same time one notices that the karyosomes are represented by regular, delicate, vacuolated vesicles, one of which is usually larger than the others. During this stage, and indeed for some time previous to it, the outline of the nucleus in fixed specimens is very irregular. This is due, I believe, to the nature of the contents

of the cyst—a large, relatively soft nucleus with a very delicate wall surrounded by a mass of hard food-granules with very little cytoplasm among them.

In giving an account of the first division of the nucleus it is unwise, I think, to do more than indicate the different phases found and to describe these as separate stages. No one has yet been so fortunate as to obtain in any Gregarine a series of figures complete enough to give a detailed description, and no little obscurity is caused by glossing over gaps instead of admitting them.

Prophases.—I have found only five early stages in the first division. They are shown in Pl. 13, figs. 35–41. Pl. 13, fig. 35 shows a nucleus in which, apart from the discrete chromatin dust, there are eight tiny particles of chromatin, each composed of one, or at most two, tiny vesicles. At a point on the periphery[1] there is seen a single small aster. It will be noted that the nuclear wall at this point has broken down. Pl. 13, fig. 36 shows the aster more highly magnified. The rupture in the nuclear membrane has allowed some of its contents to escape, and the aster is lying in the middle of this area. The aster has a dense centre with a rather diffuse outer zone.

Pl. 13, fig. 35, is the only specimen I have which shows several small chromatin vesicles, for usually there is only one large one (Pl. 13, figs. 37–40). Pl. 13, fig. 39, is typical. There is a large, very delicate chromatin vesicle seen in optical section. It will be noted that the wall of the vesicle is very thin in one hemisphere while the opposite hemisphere is thicker and vacuolated. Pl. 13, fig. 37, shows a surface view of a similar karyosome in which the thicker-walled hemisphere is budding off a vesicle. In Pl. 13, fig. 39, too, there is seen an aster which

[1] Fig. 35 has been reconstructed from seven sections. In the figure a small lighter area is seen. This represents a very small "shaving" that has been sliced off the nucleus and which is actually in an eighth section. To the right-hand side of this clear area is seen the tiny aster. The nuclear wall is there broken down. It is to this point on the periphery of the small area that I refer, not, of course, to a point on the extreme circumference of the nucleus.

is actually dividing into two, but as this is viewed from above and can only be found by focussing up and down, it is impossible to represent it in a drawing. Here again it will be noted that the aster is lying in a small mass of nuclear material which has protruded through the broken nuclear membrane.

Here I may refer to Pl. 13, figs. 38 and 40. Pl. 13, fig. 38, does not seem to fit into any normal series, for here we have two asters lying some little distance apart. They are outside the nucleus, but are actually lying in nuclear material which has evidently been extruded through the broken nuclear membrane. A rather solid-looking karyosome is seen near the nuclear membrane, and there are four food-granules lying beside and between the asters. Pl. 13, fig. 40, shows an aster lying within the nucleus and close beside a karyosome. It is, of course, impossible to say whether the aster has moved inwards from the periphery to this position or whether it originated there.

In Pl. 13, fig. 41, the origin of the chromosomes is seen. There is a single large "karyosome" in the nucleus, and near the periphery a single aster lying beside a small vesicle of chromatin from which the chromosomes are being derived. Pl. 14, fig. 42, is a more highly magnified picture of this vesicle showing the origin of the chromosomes. It will be seen that the "vesicle" really consists of one larger and two very tiny ones. Three chromosomes are lying above the vesicles. One of them is a slightly twisted beaded filament, and the other two are straight beaded filaments. At one end of each of these two there is a round globule of chromatin. The aster lies slightly beneath the chromatin vesicles. There are two vacuoles present which bulge out the nuclear wall, as if about to burst it.

Metaphase.—My preparations contain only one metaphase stage. This is figured in Pl. 14, fig. 43. The asters are large, with a rather small spindle between, on which the three globular chromosomes are dividing. Two vacuolated fragments of chromatin are lying on the achromatic figure. The division is taking place in débris of the now disintegrated nucleus.

The form of the chromosomes is to be particularly noted. In Pl. 14, fig. 42, they are seen to be filamentar, two of the filaments having thickened ends. It is evident that the chromosomes arise as filaments and then become condensed into spherical bodies. A difference in size is noticeable among the chromosomes: the right-hand pair is largest, the middle one intermediate and the left-hand one smallest.

Anaphases.—I have found several anaphases but they are all late. A typical one is shown in Pl. 14, fig. 44. The two sets of three daughter-chromosomes have retreated to the poles of the spindle and now lie close up against the asters with a stout centrodesmose connecting the two groups. The size difference in the chromosomes is still evident. It will be noted that as the daughter-chromosome groups have moved apart the spindle has lengthened so that the asters now lie farther apart than in the metaphase.

Telophases.—In the late anaphases a vacuole can be seen forming round the chromosomes, and this is undoubtedly the first sign of the reconstruction of the nuclei. Unfortunately my preparations show no stages between these late anaphases and the late telophase shown in Pl. 14, fig. 45, in which the two daughter-nuclei are fully formed. In each there is a single karyosome and a small amount of chromatin arranged in tiny particles on the nuclear wall and on the linin network. The distance between the two daughter-nuclei is now very considerable. All trace of the primary nucleus has disappeared and the nuclei lie freely among the food-granules. In these young nuclei the asters are still large. Pl. 14, fig. 46, shows an older nucleus from the first division. It has increased greatly in size, those shown in Pl. 14, fig. 45, being about 5 μ in diameter while this one is about 8·6 μ, but the aster is relatively smaller and much more condensed. It persists through the resting stage in this somewhat concentrated form on the outside of the nucleus.

6. The Later Nuclear Divisions.

Second and third divisions are not abundant in my prepara-

tions. Pl. 14, figs. 47 and 48, show a metaphase and an anaphase of the second division. Division is taking place in a little web of cytoplasm stretched between the rays of the asters. The three chromosomes are still almost spherical bodies. Three stages of the third division are represented in Pl. 14, figs. 49 to 51—an equatorial plate, an early anaphase, and a late anaphase respectively. The chromosomes are filamentar in the first two, but in the third are stumpy rods. The asters are very large and striking. Three chromosomes are present throughout.

Of the fourth and fifth divisions I have very many stages although the early prophases are rather rare. The aster divides into two (Pl. 14, fig. 52). The chromatin becomes divided into fine particles which distribute themselves over the linin network (Pl. 14, figs. 53–55). Pl. 14, fig. 53, is peculiar. The chromatin seems to be given off from the karyosome, travelling outwards from it along a very definite line. The very loose and irregular spireme which is thus formed (Pl. 14, fig. 55) condenses and segments to form three filamentar beaded chromosomes (Pl. 14, fig. 56). Fibres from the two asters invade the nucleus and come into relation with the three chromosomes (Pl. 14, fig. 57). A spindle is thus formed, with the chromosomes on the equatorial plate, and the nuclear wall disappears (Pl. 14, fig. 58). The three chromosomes split longitudinally (Pl. 14, fig. 59), and then move along the spindle towards the poles (Pl. 14, fig. 60), while at the same time the two asters draw apart (Pl. 14, fig. 61). When the chromosomes reach the poles of the spindle they move right to the heart of the asters and a vacuole begins to form round them (Pl. 14, fig. 62). The chromosomes shorten and become thick at the ends as they lie within the vacuole, and ultimately give rise to several small round chromatin masses (Pl. 14, fig. 63). Finally the greater part of the chromatin becomes concentrated in a single karyosome, a small amount being distributed in tiny granules over the nuclear wall and on the linin network (Pl. 15, fig. 64). A well-marked centrodesmose persists for

some time during the telophases, but ultimately the daughter-nuclei lose all connection with each other, the aster persisting as in the case of the first division (Pl. 15, fig. 65).

Owing to the manner in which the spindles lengthen during division, the nuclei, as division progresses, become scattered all through the body of each parasite. After about the fifth or sixth division the nuclei begin to migrate towards the periphery.

H. The Peripheral Divisions Preceding Gamete Formation.

The migration of the nuclei to the periphery is accompanied by the formation of a peripheral zone of cytoplasm free from food-granules. The nuclei on reaching this zone continue dividing rapidly. At first the divisions are similar to the earlier ones (Pl. 15, fig. 66), with the exception of the fact that the nuclear wall persists longer. Three chromosomes are present at each division, as before. The nuclei lie at the very edge of the cytoplasmic zone. As divisions proceed the asters disappear, and cones of attraction, into which the daughter-chromosomes run and there reconstruct the daughter-nuclei, are formed, projecting nipple-like from the periphery (Pl. 15, figs. 67 and 68). Later still, as the nuclei become smaller, they project almost entirely from the surface. With the decreasing size of the nuclei the chromosomes become stumpy rods (Pl. 15, fig. 69), and finally little spheres (Pl. 15, fig. 70). Division now takes place entirely within the nuclear membrane. A rough spireme is formed (Pl. 15, fig. 71) which gives rise to the globular chromosomes, lying on a spindle which practically fills the nucleus (Pl. 15, fig. 72). The chromosome plates pull apart, and at the same time the spindle pushes the nucleus out into an oblong-oval shape, with the chromosomes at each end (Pl. 15, fig. 73). A division line is formed down the middle of the old nucleus and two rounded daughter-nuclei result lying close together (Pl. 15, fig. 74).

In this way the peripheral cytoplasm zone is soon furnished with a dense pile of nuclei (Pl. 15, figs. 75 and 76). In the resting nucleus the chromatin is in the form of a cap which covers its

outer pole (Pl. 15, figs. 74 and 76), the inner portion being quite clear and structureless. Each nucleus is rather more than a hemisphere, attached to the peripheral zone of cytoplasm by a flattened surface.

i. The Formation of the Gametes.

By the time this stage has been reached the partition separating the two associates has broken down. A considerable amount of the food reserve has disappeared, so that the paraglycogen granules are not so tightly packed together. The gametes are formed by the growth of the nuclei outwards from the peripheral layer of cytoplasm, the nuclei drawing out behind them a spindle-shaped body of rather vacuolated protoplasm. The nucleus of the gamete thus lies at the outer or anterior end. The posterior end remains attached to the peripheral cytoplasm zone at first by a thick neck, but as the gamete continues to grow the attachment becomes more slender, until it resembles rather a fine stalk or tail (Pl. 15, fig. 77). The nucleus becomes smaller in size, and the chromatin, which was at first in the form of a cap, becomes more distributed over the nuclear wall and linin network, which has now appeared. The anterior end of the gamete becomes pointed, but it is much more obtuse than the tapering posterior end.

When the gamete is fully formed the attaching stalk becomes exceedingly slender, and, severing its connection with what remains of the peripheral cytoplasm, forms a short, slender "tail"—it can scarcely be called a flagellum (Pl. 15, fig. 78). The fully-formed gamete is club-shaped, with its nucleus lying in the pointed anterior end. The nucleus is large, and contains a considerable amount of chromatin arranged in granules round the periphery and on an achromatic net. The gametes of the two associates appear to be exactly alike.

j. The Union of the Gametes.

The gametes now unite in pairs. It may be noted that it appears as though there was a migration of gametes from one

associate into the other, because conjugating gametes are found more abundantly at one end of the cyst than at the other. They first become united by their cytoplasm, later drawing in the tail portion and becoming rounded up (Pl. 15, figs. 79 and 80). Complete fusion of the cytoplasm then takes place, resulting in the formation of a zygote with the two gamete-nuclei lying in its cytoplasm (Pl. 15, fig. 81). The chromatin granules of the nuclei then draw away from the nuclear membranes and collect in the middle of the nuclei (Pl. 15, fig. 81), which at first lie close together, but ultimately fuse. Within the zygote nucleus thus formed the two little clumps of chromatin granules are at first separate (Pl. 15, fig. 82), but later they unite to form a large mulberry-like karyosome (Pl. 15, fig. 83) which contracts subsequently to form a more compact body (Pl. 15, fig. 84). The zygote is now a sporoblast.

K. The First Division (Reduction Division) of the Sporoblast Nucleus.

The karyosome commences to break up. Round particles are given off from it, which seem to move outwards along the achromatic strands towards the periphery of the nucleus (Pl. 15, fig 85). The chromatin particles become arranged on a long thread coiled round the inside of the nuclear wall (Pl. 15, fig. 86). The particles are at first of two sizes—large particles and much smaller ones. The chain then begins to contract towards the centre of the nucleus and the particles become more uniform in size (Pl. 15, fig. 87), until finally a spireme is formed like a coiled string of beads (Pl. 15, fig. 88). The spireme contracts into a tangled knot towards one side of the nucleus, which is frequently drawn out into a long oval at this stage (Pl. 15, figs. 89–91). Then the knot begins to open out (Pl. 15, fig. 92). As the tangle becomes less obscure one can see that the spireme during this synapsis has become divided into six chromosomes (Pl. 15, figs. 93–95). These are as a rule rather difficult to make out, Pl. 15, figs. 93 and 94 being typical. Pl. 15, fig. 95, in which the chromosomes are widely separated, is not common. The chromosomes are in two sets of three—two

long, two intermediate, and two short, the last two pairs being very nearly equal in size.

A very faint indication of a spindle arises within the nucleus (Pl. 15, fig. 96), and the chromosomes separate into two homologous groups of three, and then move to the opposite poles of the spindle (Pl. 15, fig. 97). The daughter groups move apart until they reach opposite poles of the sporoblast (Pl. 15, fig. 98), and at the same time the chromosomes shorten and thicken. A vacuole arises around them, and two daughter nuclei are reconstructed, the chromosomes condensing to form a large karyosome in each (Pl. 15, fig. 99).

L. The Later Divisions within the Spore.

Owing to the small size of the nuclei the final divisions in the sporocyst are difficult to make out in their earlier stages. The two daughter-nuclei form spiremes by the breaking up of the karyosomes (Pl. 15, fig. 100), and then give rise to three chromosomes which divide to form daughter-groups (Pl. 15, figs. 101–103). The chromosomes are short rods. Four daughter-nuclei are reconstructed (Pl. 15, fig. 104). These in turn give rise to rough spiremes (Pl. 15, fig. 105), each forming three globular chromosomes which again divide (Pl. 15, figs. 106-108), and eight nuclei thus result.

The sporozoite nuclei are formed from the eight nuclei of the sporoblast. The chromatin is first peripherally placed (Pl. 15, figs. 110 and 111)[1], but it becomes concentrated more and more into two granules which lie one at each end of the oval nucleus (Pl. 15, figs. 109 and 110). The cytoplasm in the sporocyst becomes divided, in the usual way, into eight sporozoites round the eight nuclei.

The formation of the spore-wall is difficult to make out in stained preparations mounted in balsam. It is evident in the late eight-nucleate cyst and appears to begin to form during the last division.

[1] These two figures, containing only four nuclei and rather smaller in size, are "chips" of rather longish oval sporoblasts in the eight-nucleate stage.

Part II.—The Nuclear Phenomena described in other Gregarines, considered in the Light of the Foregoing Observations on Diplocystis.

A. General Remarks.

Having described the behaviour of the nuclei during the life-cycle of Diplocystis schneideri in so far as I have been able to investigate it, I wish now to compare my findings with those of other workers in the same field. Unfortunately most of the work on gregarine life-histories is extremely scrappy. So far as I know, no worker has up to the present attempted to follow in detail the behaviour of the nucleus through every stage in the life-cycle of any Gregarine. On some points, such as the question of the similarity or dissimilarity of the gametes, our knowledge is very full, but on the majority of questions that seem to me to be of interest and importance the information available is lamentably scanty. I shall therefore attempt to combine all the scattered observations, and so construct a more or less connected account of the gregarine nuclear cycle.

In reviewing this work it will be unnecessary to pay any attention to the papers published prior to 1899, in which year Siedlecki's most excellent account of Lankesteria ascidiæ appeared. This investigation outlined the course of gregarine development in a masterly fashion and left only the details to be filled in. During the twenty succeeding years many papers on Gregarines have been published, but with certain outstanding exceptions they have not advanced our knowledge of the subject from the position in which Siedlecki left it. I have read, I believe, all the papers published on gregarines during these years, and if I do not refer to some it will be because they contain little or nothing of importance.

As the Schizogregarines are so very imperfectly investigated I have confined my attention to the Eugregarines. These form a very homogeneous group, and I believe that

when they have been more fully studied their life-cycles will be found to be of a very uniform type. At all events a comparison of the extremely imperfect records at present available shows far more of resemblance than of difference.

B. The Early Development of the Nucleus.

Very little attention has been paid by most workers to the early stages of gregarine development. Léger and Duboscq (1902 and 1904) have investigated a number of different forms, but no one of them has been studied in much detail. In all cases the sporozoite nucleus is of the type described for D. schneideri, with peripheral chromatin concentrated in two, usually polar, caps. A single central karyosome is subsequently formed in a way which is not clearly described, but the final result is that the peripheral chromatin disappears, being apparently transferred from the periphery to the karyosome. The karyosomes thus formed are frequently composed of two parts. Thus in Stylorhynchus oblongatus and Pyxinia möbiuszi there is a central more deeply-staining area, just as in Diplocystis schneideri. In Diplocystis major the same thing is found, their fig. 8 (Léger and Duboscq, 1902) being remarkably like my Pl. 12, fig. 21. In connection with this apparently dual nature of the karyosome in gregarines it may be noted that Hesse (1909) mentions certain species of Monocystis which show the same sort of thing.[1] I would call attention particularly to figs. 132 and 135 in Hesse's work, showing the nucleus of Rhynchocystis pilosa with a karyosome having in its middle "une masse irrégulière, vacuolaire, fortement colorée par le fer."

The only account which gives a detailed description of the origin of the karyosome is that of Schellack (1907) for Echinomera hispida. Here the sporozoite nucleus is unlike that of most other gregarines, its chromatin being more or less evenly distributed around the periphery. A thickening of chromatin then appears at some point on the

[1] "En générale, le karyosome est vacuolaire, et il présente à sa surface une croûte dont la coloration diffère de celle de la moelle."

periphery, and in close connection with this a small internal vesicle arises, into which the chromatin of the thickening migrates in the form of small particles. The vesicle finally becomes completely filled with chromatin and stains a uniform deep black with iron-hæmatoxylin.

The evidence before us is not abundant, but there is enough to show that in young gregarines the karyosome is not a simple structure. As a rule it contains within it a chromatin body which I have called the micronucleus. The karyosome, further, is formed within the young nucleus in a peculiar fashion. In Diplocystis schneideri a plastin nucleolus first arises, and later the micronucleus migrates into its interior. It is possible that Schellack's vesicle is also a nucleolus of this sort, and that more delicate staining would reveal that his "karyosome" is not a homogeneous, deeply-staining body, but contains, as in many other gregarines, a micronucleus which migrates into the nucleolus in small particles—as described by Schellack—from the peripheral chromatin thickening.

The true interpretation of the structure and dual origin of the karyosome cannot yet be given. I have called the chromatin body within the karyosome a micronucleus because I believe that it contains the chromatin from which the chromosomes of the first division are derived. Owing to the fragmentation of the karyosome it has been impossible in Diplocystis to follow the history of the micronucleus continuously from its origin to the formation of the chromosomes, but, as I have noted above, there is a hint of continuity in the regular chromatin vesicles which are always to be found in the nucleus among the irregular fragments of karyosome débris. An investigation of some gregarine in which the karyosome does not fragment so early or so completely as in Diplocystis would probably yield important results.

I am confirmed in this interpretation by the evidence afforded by Aggregata eberthi. This coccidian[1] has been inves-

[1] Dobell (1914) has shown that Aggregata is a coccidian and not a gregarine.

tigated in its asexual cycle in the crab, Portunus depurator, by Léger and Duboscq (1908). In this they describe the formation, in the nucleus of the young schizont, of a plastin nucleolus beside which there arises a condensation of chromatin. This karyosome, as the authors call it, makes its way inside the nucleolus and gives rise to "un véritable noyau intranucléolaire qui constituera la couche médullaire du nucléole." My friend Mr. Clifford Dobell, F.R.S., has shown me his beautiful preparations and unpublished drawings of Aggregata eberthi, and he has most kindly allowed me to make the following statement about them. The "karyosome" is formed, much as Léger and Duboscq describe it, not only in the asexual cycle but also in the young male and female forms. The most interesting point, however, is that the chromosomes are eventually formed—in both the sexual and asexual forms—from the micronucleus, which gives off into the nucleoplasm the material from which the chromosomes of the first nuclear division are directly derived.

c. The Behaviour of the Nucleus in the Later Vegetative Stages.

In most Gregarines the chromatin of the nucleus is concentrated, during the early stages, in a single karyosome. A small quantity of chromatin may be present in the form of granules on the linin network, but it is never abundant and may be absent. In many forms the karyosome fragments early in development, while in others it seems to remain unfragmented until just before the first nuclear division. The various accounts of the fragmentation or multiplication of the karyosomes differ somewhat in details, but on the whole it may be said that the process is one of budding accompanied in many cases by vacuolization. The small karyosomes thus formed seem to increase in size and give rise to others in the same fashion. Robinson (1910) has given a short account of a rather different phenomenon in

Kalpidorhynchus arenicolæ. Here the karyosome is said to be hollow and budding takes place into its interior, the young karyosomes escaping later into the nucleoplasm through a temporary micropyle. Robinson points out that Léger and Duboscq describe a similar method of budding in Aggregata,[1] but Kalpidorhynchus is the only case so far described among the Gregarines. It is an organism which would repay careful study. Schellack (1907) claims that in Echinomera hispida the karyosome multiplies in a peculiar manner: "Im Innern des grossen Einzelkaryosome die helle Grundsubstanz wieder mehr hervortritt, das vorhandene Chromatin sich zu Kugeln zusammenballt und schliesslich austritt, wobei der farblose Restkörper mehr oder weniger vom Chromatin befreit zurückbleibt." Judging by the figures, this seems to be nothing different from the disintegration by budding and vacuolization described above.

In Gonospora testiculi, Trégouboff (1918) found that at an early stage in development the karyosome was hollow, containing a clear vacuole in its centre. The contents of the vacuole were expelled into the nucleoplasm later and there gave rise evidently to a micronucleus. Léger and Duboscq (1911) have observed a similar phenomenon in Porospora portunidarum.

The karyosome in gregarines is undoubtedly the centre of many activities. For aught we know it may preside over all the vital functions of the organism, but it gives no clear indication of this. The extraordinary account given by Drzewecki (1904, 1907) of the waxing and waning of the nucleus and the corresponding building up and breaking down of amyloid bodies must be regarded with the greatest suspicion. Hesse (1909), however, has shown that "during the course of the vegetative life, the karyosome seems to be

[1] It is difficult to understand how the budding could take place in Aggregata, for the interior of the karyosome is occupied by the micronucleus. Mr. Clifford Dobell tells me that in Aggregata the buds are really formed on the outer side of the karyosome, and not internally.

the seat of numerous metamorphoses, and plays an important part in the vital manifestations of the nucleus." As far as one can judge from Hesse's descriptions and figures the karyosome does not appear to be altered to any very great extent during most of these changes, evidently a change in staining reaction being the most noticeable.

In the early stages there is, however, one important period during which—in Diplocystis schneideri at all events—the karyosome gives very clear proof that it is connected closely with an important vital function. I refer to the formation of reserve food-granules. While the karyosome is single and unfragmented there are no food-granules present in the cytoplasm; but soon after the process of fragmentation has begun food-granules begin to appear round the nucleus. In the work of Hesse (1909) evidence of a similar though less striking sort may be found. For example, in Monocystis agilis and Stomatophora coronata granules are given off by the karyosome, and they seem to make their way out of the nucleus into the cytoplasm and there play an important part in the synthesis of food-granules.

These phenomena must not be confused with the so-called "chromidia" which have been frequently described in the vegetative forms of various gregarines. By a careful investigation of Stylorhynchus longicollis and Stenophora juli, Comes (1907) has shown that these bodies are not nuclear in origin, but are formed in the cytoplasm as the result of various metabolic processes.

D. The Behaviour of the Nucleus during Reproduction.

Whatever the behaviour of the karyosome may be during the vegetative life of gregarines, there is no little uniformity in the processes which precede the reproductive phases. This period is practically always introduced by the disintegration of the karyosome or karyosomes. There is thus formed within

the nucleus a large quantity of chromatin débris in fine particles and small, usually vacuolated, fragments. In all cases which have been adequately investigated an achromatic figure is formed and division proceeds in the ordinary mitotic fashion.

It may be well at this point to note that there are a few cases on record in which mitosis is said not to occur. All of these accounts are obviously founded on the misinterpretation of poor material, but as they have been made the occasion for the revival of the "generative chromidia" hypothesis in gregarines it will be necessary to consider them briefly. Berndt (1902) describes in Gregarina cuneata, G. polymorpha and G. steini a process whereby the chromatin becomes distributed round the periphery of the nucleus in a very finely granular form. The nucleus itself becomes very irregular in outline ("flame structure"). Its membrane disappears, and the chromatin is then set free in the cytoplasm in little masses which migrate to the periphery of the organism, multiplying in a "primitively mitotic" fashion as they go. Kuschakewitsch (1907), for the same gregarines, describes a still more wonderful history, in which the nucleus seems to dissolve away completely, and there then arises round the periphery of the cyst a layer of cytoplasm which stains deeply with chromatin stains, and within which grains of chromatin appear and finally give rise to many nuclei. An inspection of the figures which illustrate these papers is sufficient to convince one that all the material on which these results are based was extraordinarily badly fixed and stained. The mere fact that the "flame structure" of the nucleus was taken to represent a normal stage in development seems to me sufficient to make one regard the work with doubt; for despite the fact that Drzewecki (1903) claimed to have seen this appearance in a living nucleus, I believe it is produced by the action of fixatives on the nuclear wall, which becomes very thin prior to disintegration. As Cuénot (1901) has pointed out: "Les Grégarines sont très difficiles à bien fixer, le noyau se déforme avec la plus grande facilité et prend un

aspect irrégulier (noyau flammé de Wolters)." If a badly fixed or degenerate nucleus is taken as an important stage in development, disaster is bound to follow.

Fortunately Léger and Duboscq (1909) have investigated a number of species of the genus Gregarina, including G. polymorpha, and in every case they have seen early nuclei dividing by mitosis. Further, we have Schnitzler's (1905) account of Clepsydrina (Gregarina) ovata, in which he describes a typical mitosis for the first nuclear division. It is unlikely that within the same genus there would be two totally different methods of forming the gamete nuclei.

Swarczewsky (1910) describes, in Lankesteria sp., a process of gamete-nuclei formation which is almost identical with that described by Kuschakewitsch for the meal-worm gregarines. The figures published with the paper are quite as remarkable as those of Kuschakewitsch, and when one learns that the material was obtained from Turbellarians collected by an expedition to Lake Baikal—evidently no special care having been taken in fixing the worms so as to preserve adequately the contained parasites—one is not surprised at the unfortunate results. It is only astonishing that such material should have been regarded as adequate for an investigation requiring the most careful cytological technique. Here again we are fortunate in having a good account of the behaviour of the nucleus in another member of the same genus. Siedlecki's (1899) account of Lankesteria ascidiæ traces the nuclei from the first division to gamete formation, and in every stage typical division figures are found.

Swarczewsky (1912) has attempted to argue that all gregarines form their gamete nuclei by a process of chromidia formation, the difference between the findings of Kuschakewitsch and Swarczewsky and those of other workers being "more of a quantitative than of a qualitative character." On the one hand many nuclei are formed from a chromidium; on the other only one nucleus is so formed. As we have seen,

the supposed origin of the many nuclei from a chromidium is the result of studying degenerate and badly-fixed material, while the "chromidium" from which the single nucleus is supposed to be derived is merely nuclear débris. The question of generative chromidia—at least for the Eugregarinæ—does not really exist. It has been based on error and ought never to have been raised.

Putting on one side, then, these obviously faulty observations,[1] we are left with some nine or ten cases in which a typical mitosis has been observed. The first nuclear division is probably the most difficult stage to study in a gregarine life-cycle. It is extremely difficult to find sufficient material to illustrate the various nuclear phases leading up to and culminating in the division of the primary nucleus; and when any stages are found they are frequently obscured by the débris from the disintegrating karyosomes. In consequence of this, very imperfect accounts of the first nuclear division are given by different workers. Further, when material is scanty there is always the temptation to make the most of it, and to attempt to construct a "series" from only two or three doubtful stages. Palæontologists may be able to reconstruct an extinct monster from a few fragments of skeleton, but it is not advisable for protozoologists to adopt a like method. A gregarine life-cycle is not known from the nuclear divisions which precede gamete formation, nor can the division of a nucleus be adequately described when all the available material shows perhaps only one or two very problematic prophases and an odd metaphase or late anaphase. I am far from under-estimating the difficulties which

[1] Another erroneous case may be mentioned. Woodcock (1906), in his paper on Cystobia irregularis, believes that the early divisions are amitotic. In proof of this a very poor and meaningless figure is published showing two bodies, presumably nuclei, connected by a short strand; and we are told that in an individual containing "about thirty daughter-nuclei of the fourth generation," one nucleus of the previous generation was thought to be in the last phases of an indirect division. Such evidence does not carry conviction.

attend the study of gregarine life-histories, but I cannot help thinking that if a larger amount of better material had been carefully examined in certain cases, very different results would have been obtained.

The only accounts of the origin of the first division figure that are at all satisfactory are for members of the Acephaline gregarines. It is extremely unfortunate that we know so little of the early development of the Cephaline forms. For these there are but five accounts of the early nuclear phenomena—Clepsydrina (= Gregarina) ovata by Schnitzler (1905) and Schellack (1912), Echinomera hispida by Schellack (1907), Nina gracilis by Léger and Duboscq (1903 and 1909), and Metamera schubergi by Duke (1910). Of all these examples, moreover, only in Metamera schubergi has the investigator been able to find more than one or two stages. Thus Schnitzler figures two very early prophases and two metaphases in G. ovata; Schellack, for E. hispida, one very early prophase and one metaphase, while for G. ovata he merely confirms and corrects the scanty observations of Schnitzler; Léger and Duboscq four "stages" in N. gracilis, the interpretation of which is doubtful, and which will have to be considered later.

On the other side, some of those who have worked on the Monocystid gregarines have been more successful, and have obtained, if not complete series of stages, at all events a fair number of representative ones. For practically all our knowledge of the origin of the achromatic figure and the chromosomes of the first division we shall be compelled to resort to the members of this group.

Let us first consider the origin of the achromatic figure. Following the disintegration of the karyosome the next step in the process of division is the appearance of an achromatic figure. As a rule this arises at the periphery of the nucleus as a single, round, achromatic body or aster. Mulsow (1911) always found two asters in Monocystis rostata, and thought it probable that they arose independently and not by the division of one into two; but it seems most likely that

he failed to discover the earliest stages. At all events, Cuénot (1901) and Brasil (1905), who also studied species of Monocystis, found a single aster which later divided into two. In Diplocystis schneideri this is also the case, and there is a hint of the same sort of thing in Echinomera hispida, as described by Schellack (1907). In Metamera schubergi Duke found that a large achromatic mass appeared inside the nucleus, and gradually became striated and formed a spindle without asters, but all the early nuclear development of this gregarine seems very peculiar. In the various members of the genus Monocystis the asters seem to arise on the outside of the nuclear membrane. In Diplocystis schneideri the origin of the aster seems to be at the periphery of the nucleus, near the spot where the nuclear wall begins to break down, but always in nuclear material. It seems probable that the achromatic figure is always of nuclear origin, for in the cases in which it does not arise inside the nucleus or in nuclear material it is closely applied to the outside of the nuclear wall.

There has been some discussion as to the presence or absence of a centrosome or centriole during the first nuclear division. The question is one to which I myself do not attach much importance, for I believe that "the centrosome is but a subordinate part of the general apparatus of mitosis and one which may be entirely dispensed with" (Wilson, 1906). The evidence on this point is most conflicting. Some authors—e.g. Hesse (1909) for Stomatophora coronata, and Léger and Duboscq (1902) for Stylorhynchus longicollis—have supposed that a granule found in the cytoplasm near the nucleus, even in very young individuals, represents a centriole; but I think the assumption has little to be said for it. These deeply-staining grains have not been shown to take any part in the division of the nucleus, and behaviour during division is, after all, the only test of a centriole. In the descriptions and figures which we now have of the actual division stages, nothing definite is to be found. Thus Mulsow (1911) could demonstrate no centrosome in the asters of

Monocystis rostrata; indeed, the presence or absence of an aster seemed to him to depend on fixation, so that one might even doubt the quality of his material and cytological technique. In Diplocystis schneideri I have found no trace of a centrosome, and in the later divisions at the periphery even asters are absent. On the other hand, in Diplocystis major Cuénot (1901) found large, massive-looking centrosomes. Schnitzler (1905) in Clepsydrina ovata found centrosomes but no asters. Brasil (1905), unlike Mulsow, found very definite centrosomes in all species of Monocystis which he studied. Finally Duke (1910) found in the first divisions of Metamera schubergi neither aster nor centrosome, although in the later divisions both were present. On the whole it seems to me most probable that in gregarines the centrosome is no very definite organ, and its presence in preparations may be dependent to a great extent on the fixative and stain used.

This much may then be said about the achromatic figure. It arises on the periphery of the nucleus, and is most probably nuclear in origin. The original single aster or centrosome or attraction cone—whatever it may be, and whatever one cares to call it—divides into two, and so forms a spindle. The substance of the achromatic figure persists from generation to generation in the form of a daughter-aster, which remains usually on the outside of each daughter-nucleus during its period of reconstruction and rest, and gives rise in turn to two daughter-asters and a new spindle at the next division.

The origin of the chromosomes is a much more interesting and important question. Léger and Duboscq (1909) express the opinion that the method of formation of these varies according to the species of gregarine, there being at least two possibilities: (1) the first division figure may derive its chromosomes directly from the chromatin of the primary nucleus, or (2) a micronucleus may first be formed from which the chromosomes arise later. Such evidence as we possess does not, I think, support this second view. As we shall see, the few cases which have been at all adequately studied show

the formation of the chromosomes from a certain portion of the chromatin of the primary nucleus, without the intermediate formation—at this stage—of a micronucleus.

Let us first consider the "micronucleus." The word has been used very loosely. Cuénot (1901) originally gave the name "micronucleus" to "des grains ou de courts filaments groupés en un petit amas" in Monocystis; but when writing of Diplocystis he applied the same term to a fully organized small nucleus. It is, I believe, to this latter kind of "micronucleus" that Léger and Duboscq apply the name, for they both figure and describe in Nina gracilis "une véritable noyau très claire." There are only two cases of this sort recorded—the two mentioned above. Schellack (1907) compared the vesicle which he found in the prophases of the first division of Echinomera hispida to the "micronucleus" of Cuénot; but he must have referred to the "micronucleus" of Monocystis and not to that of Diplocystis, for the figure published does not show a completely organized small nucleus. Later (Schellack, 1912) he refers to it as "eine Art Kleinkern." I shall discuss the "micronucleus" of Schellack below. In the Diplocystis from the cricket which Cuénot describes, after some very peculiar "prophases" evidently four stages were found—a doubtful metaphase, a late anaphase, a single small nucleus (the "micronucleus"), and a stage with two small nuclei. Cuénot was of the opinion that the "micronucleus" arose in some fashion in the cytoplasm, then followed the metaphase and anaphase, and finally the two small nuclei resulted. At the same time he recognized the possibility that the "micronucleus" might be merely one of two daughter-nuclei, the other having been knocked out of the section by the knife. I feel certain that this is the correct interpretation. Léger and Duboscq (1909) describe a similar "micronucleus" for Nina gracilis. Here, again, only four stages were found —or, at all events, figured. The first shows the primary nucleus containing a small clear area, within which is a little group of chromatin particles—probably really the

micronucleus. The second shows the primary nucleus disintegrated, with a small stellate area among the débris within which are a few regularly-shaped portions of chromatin —presumably chromosomes. The third is extremely doubtful, and is merely called "formation du micronucléus" (fig. 11). I confess to being able to make out little or nothing in this figure except a rather clearer circular area in the nuclear débris. The fourth figure shows a perfectly formed nucleus with a centrosome. The interpretation seems to me to be the same as for Cuénot's "micronucleus"; it is merely one daughter-nucleus derived from the first division, the other having been overlooked or having dropped out of the section. The figure showing the stellate area with chromosomes is probably really an equatorial plate viewed from one pole of the spindle. There is a point in connection with these so-called "micronuclei" which neither Cuénot nor Léger and Duboscq seem to have noticed. It is this: Supposing these nuclei really do represent a stage between the disintegration of the primary nucleus and the formation of the chromosomes, then before the chromosomes can be derived from such a typical nucleus a series of prophases would have to be gone through. But these have never been found by anyone: and since all these stages are missing, the evidence in favour of the "micronucleus" as described by Cuénot and Léger and Duboscq does not seem to me to be satisfactory. All these cases are thus possibly misinterpretations caused by the lack of sufficient material. It is very probable that the origin of the chromosomes in gregarines takes place in a general way after the fashion now to be described.[1]

As we have seen above, in practically all gregarines the karyosome or karyosomes become broken up into many fragments, some very minute and some larger. It is from some of these that the chromosomes of the first equatorial

[1] Prowazek (1902) claimed to have seen the nucleus of Monocystis open and give off its contents "in an indistinct striated form" into the cytoplasm. This gave rise to a "micronucleus." As no figures are published these observations must be regarded with the greatest suspicion.

plate are derived. Most unfortunately very little is known about this extremely interesting stage. Brasil (1905) was the first to publish any observations on the point. In Monocystis he found in his "third type of division" that a round, vacuolated portion of a karyosome gave off numerous small particles which at once formed a spireme and then gave rise to the chromosomes. There is one figure (fig. 1[1]) in Robinson's (1910) account of Kalpidorhynchus arenicolæ which is very suggestive of a spireme, but the whole description is too sketchy to make much of. Mulsow (1911) was fortunate enough, in his work on Monocystis rostrata, to find several stages in the formation of the chromosomes. Here, again, a spireme is formed from what appears to be a round, vacuolated portion of karyosome, the spireme being formed from tiny, rounded pieces of chromatin. A sort of "synapsis" occurs, and the chromosomes form a tangled ball on the plate of the spindle, after which division evidently occurs. In Diplocystis schneideri I have found only one example of the formation of the chromosomes, and here again they are seen to come off in the form of beaded threads from a rounded and vacuolated portion of karyosome, very like the original micronucleus formed in the nucleolus of the young forms. It seems to me almost inconceivable that any fragment of chromatin can form chromosomes in this way. There must surely be some particular portion of chromatin that has this function. As I have already said, I cannot help believing that if it were possible to follow the micronucleus of Diplocystis schneideri through all the nuclear changes from its formation in the youngest parasites up to the stage of the final break-up of the primary nucleus, a complete chain might be established between the chromatin of these early stages and the chromatin which forms the chromosomes. It

[1] Duke (1910) has a figure (fig. 15) of Metamera schubergi which is rather like this one, and might almost suggest a spireme; but he failed to find any chromosomes. The whole account of the first nuclear division is very peculiar and difficult to understand. It is quite unlike that of any other gregarine.

is at least significant that in those few cases in which the formation of chromosomes has been seen, they all arise in the same fashion from a small chromatin structure of approximately the same form.

I believe one is justified in calling this more or less organized portion of chromatin a micronucleus. There are two cases—apart from the erroneous two already referred to—in which micronuclei have been described, but in which the connection between them and the chromosomes has not been found. I refer to the already mentioned cases of Echinomera hispida and Gonospora testiculi described respectively by Schellack (1907) and Trégouboff (1918). The "micronuclei" in both these differ very much from those of Nina gracilis and Diplocystis major; they are merely tiny vesicles containing small fragments of chromatin. In Echinomera Schellack was able to see the formation of the karyosome, and his figures of the early stages show the "karyosome" to be extremely like the later "micronucleus." Might it not be that, as in Diplocystis schneideri, the micronucleus is formed very early in the life of the organism and remains hidden within the karyosome until just before reproduction, when the disintegration of the karyosome brings it to light again? In Gonospora the micronucleus seems to be formed early in the life-history, being expelled from the karyosome as already described. Before the onset of the first division the micronucleus becomes completely organized, and forms a tiny vesicle very like that of Echinomera. Most unfortunately the formation of chromosomes has not been observed in either of these forms.

At this point I propose to review the rest of our knowledge of chromosomes in gregarines, although in some cases, to which reference will be made, the chromosomes were not studied until the later divisions. I wish first to refer to the chromosome numbers found in different species. The more important of the observations recorded in this connection have been combined in the following table. In many cases no counts have been made by the investigator, but I give in

a separate column the numbers shown in the published figures. Even when counts have been made I have also given the numbers shown in the figures, for the two do not always correspond.

Species.	Investigator.	No. of chromosomes given.	No. shown in figures.
Diplocystis major and minor	Cuénot (1901)	—	3
Diplocystis schneideri	Jameson (1915)	3	3
Gregarina munieri .	Léger & Duboscq (1909)	—	3
Gregarina ovata . .	Schellack (1912)	3	3
Uradiophora cuénoti .	Mercier (1912)	—	3
Kalpidorhynchus arenicolæ	Robinson (1910)	4	4, many or 3
Monocystis (3 species mixed)	Cuénot (1901)	—	Many, 4 or 3
Monocystis, "2nd type"	Brasil (1905)	—	4 or 5
Monocystis, "3rd type"	,, ,,	—	4–6
Monocystis pareudrili	Cognetti de Martiis (1911)	4–5	4–5
Stenophora juli . . .	Trégouboff (1914)	4, seen only at "reduction"	4
Stylorhynchus longicollis	Léger (1904)	4	4
Echinomera hispida .	Schellack (1907)	5	5
Nina gracilis . .	Léger & Duboscq (1903)	5	5
Urospora lagidis .	Brasil (1905a)	8	8
Monocystis rostrata .	Mulsow (1911)	8	8 (& ? 4)
Monocystis, "1st type"	Brasil (1905)	—	Many
Metamera schubergi .	Duke (1910)	No definite chromosomes.	

One cannot fail to notice in this list the remarkable number of gregarines which have an odd number of chromosomes. Léger and Duboscq (1903) were the first to draw attention to this point in their work on Pterocephalus nobilis (= Nina gracilis). There they found five chromosomes, four short ones and one long one, which they called the axial or unpaired chromosome, and which they found formed the karyosome of the daughter-nuclei after division. Schellack (1907) found the same sort of thing in Echinomera

hispida. The fifth chromosome was longer than the others and formed the karyosome. He compared it with the accessory chromosomes in insects. Léger and Duboscq (1909) returned to the same subject later and showed that in Nina gracilis the unpaired chromosome forms the karyosome only in part. They also pointed out that Schellack's comparison between the unpaired chromosome in gregarines and the accessory chromosome in insects was not satisfactory, and compared it in turn to a "nucleolar formation." As we shall see later, there is a simple explanation of the odd chromosome numbers found in the gregarines. It seems, indeed, that the "unpaired chromosome" differs in no way from the others except in size, and the size difference may amount to little or nothing; for in Gregarina ovata, in which Schellack (1912) discovered another "unpaired chromosome"—three chromosomes are present—the "odd chromosome" hardly differs at all in size from the two others.

A word may be said about the form of the chromosomes. As a rule they are very definite filamentar, rod-shaped or globular bodies. Often indeed all three different forms are found in one species at different stages of the life-history, as we have seen in Diplocystis schneideri. Occasionally, as in Brasil's (1905) "first type of division" in Monocystis, the chromosomes are represented as many small grains of chromatin. The case of Metamera schubergi, investigated by Duke (1910), is an extreme one of this sort. Here chromosomes are apparently absent, and it is suggested that the chromatin is carried along the spindle-fibres in "discrete particles." The method of nuclear division in this gregarine is so peculiar that its further study is desirable.

When the achromatic figure and the chromosomes have been formed the division takes place in the ordinary mitotic fashion. In many cases the two daughter-groups of chromosomes move apart a considerable distance by the lengthening of the spindle-fibres. In this way the nuclei of the later divisions become well distributed through the cyst. After a number of divisions have taken place all through the cyto-

plasm the nuclei migrate to the periphery of the cyst. There further multiplication may occur, or there may be only one or two peripheral divisions prior to gamete formation. In most forms the nuclei after the last division proceed to gamete formation, but in some, such as Nina gracilis and Echinomera hispida, the nuclei which are to form the female gametes migrate back from the periphery after multiplication has ceased and form a network through the body of the organism.

Léger (1904) described two types of nuclei, generative and somatic, from Stylorhynchus longicollis. Of these the somatic are the larger, and are thought to have the function of working over the reserve food stuffs and preparing them for the generative nuclei. Nuclei of these two sorts have also been described from Gregarina munieri by Léger and Duboscq (1909), Monocystis sp. by Hoffmann (1909), Stenophora juli by Trégouboff (1914), etc. Others have not observed this nuclear dimorphism, and are of opinion that the large nuclei sometimes found alongside of smaller ones are merely ordinary nuclei delayed in their division (Mulsow, 1911); and it seems clear that these nuclei which get left over and do not develop in time must be incapable of taking part in gamete formation (Brasil, 1905). At present there appears to be no good evidence to show that the nuclei within the cyst are differentiated into the two classes—somatic and generative—postulated by Léger.

E. The Search for Reduction Divisions.

The two divisions immediately preceding gamete formation have received a great deal of attention, for most workers seem to have examined these stages with particular care in the expectation of finding a reduction division after the fashion of the Metazoa. The most varied results have rewarded the investigators, but as a rule nothing really comparable with a true reduction division has been found. Before one can speak of a reduction division having taken place one must demonstrate that the number of chromosomes has been

reduced to one half by a process of indirect division. There are only two cases on record which claim to have done this. Before examining these let us note certain other nuclear processes which have been observed.

In certain cases—e.g. Stylorhynchus longicollis, Léger (1904), Echinomera hispida, Schellack (1907)—a granule of chromatin has been found expelled from the nucleus prior to gamete formation. Schellack (1907) believed that this must represent some sort of reduction process, especially in view of the fact that E. hispida had an odd number of chromosomes. Hoffmann (1909) found similar granules in Monocystis, but did not believe that they had anything to do with reduction. Brasil (1905) found that after conjugation the nucleus of Monocystis expelled some granules of chromatin into the cytoplasm, but thought they were of a vegetative nature. Thus Schellack's interpretation of the expulsion of the granules from the nucleus is not supported by any others, and, indeed, he himself does not seem to be greatly intrigued by it.

In 1904 Paehler described from Gregarina ovata a process which outwardly closely resembled the formation of polar bodies in the metazoan egg. Later Schnitzler (1905), who worked on the same species as Paehler, described this process still more fully. The gamete nuclei become surrounded by cytoplasm and break free from the parent organism. A centriole appears, which presumably divides into two, and a spindle is formed. "In a typical karyokinesis two daughter-plates are formed, of which one remains in the sporoblast and gradually rounds up to form the sporoblast nucleus, while the other becomes condensed and cut off as a reduction body." Only one division was said to take place, and no chromosomes were counted. From Schnitzler's figures it would seem as if three chromosomes were dividing into two groups of three; and although Schnitzler gives four as the chromosome number in the preceding nuclear divisions, his figures of these also frequently show three only. Later Léger and Duboscq (1909) describe a similar happening in Gregarina munieri,

but here two "reduction" divisions take place, the "polar body" dividing into two. Again there is no evidence of a reduction in chromosome number, and although no chromosome counts are given, three chromosomes are shown in the figures. On the whole this seems to be a rather complex method of expelling a chromatin granule similar to those described in the previous paragraph. Schellack (1912) has re-examined G. ovata with great care, and he has found that the number of chrosomones is three, and not four as Schnitzler had said. Further, although he examined the necessary stages with the greatest care, in order to observe the "reduction" phenomena, he was unable to confirm the findings of the previous workers. He says, indeed, that the karyosome expelled a granule which made its way into the cytoplasm; but nothing more happened. He found no "polar body." This type of "reduction" has been found only in the genus Gregarina, and—which is noteworthy—only in species which have an odd number of chromosomes. It is difficult to understand how the three "somatic" chromosomes could be reduced (halved) in this way. Moreover, quite apart from the fact that no true reduction in chromosome numbers has been established, the phenomenon in question does not seem to be of constant occurrence. What the significance of the expulsion of a small portion of chromatin may be I cannot suggest; but I am inclined to believe that, like the previously described processes in Stylorhynchus and Echinomera, it has more to do with the vegetative than the reproductive side of the gamete's existence.

There remain two cases of reduction that are important because in them the chromosomes have been counted. They will have to be considered in detail. Mulsow (1911) described what he believed to be a reduction division in Monocystis rostrata, a new species which he found in the seminal vesicles of Lumbricus terrestris. This species was said to have eight chromosomes. There was only one reduction division, the last division before gamete formation. At this division eight chromosomes appeared as usual when the

spireme became segmented. They were thought to be associated in pairs but not actually united, as, on analogy with a metazoan reduction, Mulsow expected. The eight chromosomes then separated into two groups of four, a daughter-group going to each pole of the spindle. It has already been pointed out (Dobell and Jameson, 1915) that Mulsow was probably dealing with two species of Monocystis—one with eight chromosomes, the other with four—and that what he observed was not an instance of reduction but one of confusion.

In the first place it must be noted that Hesse (1909) has recorded six species of Monocystid gregarines from Lumbricus terrestris, and that Mulsow's new species makes a seventh. Several of these are very common, and mixed infections are of regular occurrence. To expect to find always a pure infection would seem to be folly. Even when the vegetative forms are easily enough distinguished the cysts are usually extremely alike. Further, we have already seen that four is not an uncommon number of chromosomes in various species of Monocystis. Mulsow himself, it seems to me, has supplied evidence which points to a mixed infection. He found always a certain number of division figures which were quite unlike those of Monocystis rostrata. He has given samples of these in his figs. 54–57. His explanation of these is that they are due to bad fixation, but at all events figs. 54 and 56 seem to be excellently fixed, and I would suggest that the differences may be due rather to the fact that the different spindles really belong to different species of Monocystis. Even in the figures illustrating the normal life-cycle of Monocystis rostrata there are a number of suggestive discrepancies. Thus in figs. 2 and 20, which show the origin of the chromosomes, only four chromosomes are figured. Of course it is conceivable that four more might form later; but this would be extraordinary, and it is far more probable that four is the complete number. It may be noticed also that the chromosome pairs shown in fig. 46 are not really pairs at all, but eight distinct and

different chromosomes. Furthermore, if the figures showing the prophases—figs. 38–46—are compared with those —figs. 48–53—which are supposed to show the actual reduction, it will be seen that the chromosomes shown in the prophase figures are quite different from those shown in the division stages. Finally, in the reduction division itself, figs. 48 to 50 show the four daughter-chromosomes moving to each pole of the spindle, but still connected together at their ends—not eight chromosomes separating into two groups of four, but four chromosomes apparently dividing into two daughter-plates of four each. Mulsow noted the connection of the daughter-groups at this stage, but again blamed the fixation. The connection together of chromosomes by their ends or by delicate chromatin strands is a well-known feature of dividing chromosomes, but I doubt if any amount of bad fixation would bring it about in fully individualized chromosomes which were merely separating into two lots.

The more one examines Mulsow's case of "reduction" the less is one satisfied with it. To me the evidence seems to point strongly to the confusion of a species having four chromosomes with one possessing eight. If Mulsow's account of this division is correct, I can but conclude that it is a special form suited only to those species whose "somatic" chromosome number is even.

The other case in which a reduction in the number of chromosomes is said to have been observed is that of Stenophora juli, described by Trégouboff (1914). The "reduction" is after the fashion described above for Gregarina, but "un peu spéciale." No reduction could be found during the formation of the microgametes on account of the unfavourable material; but when formed they were seen to contain "two chromatin bodies placed side by side—certainly the chromosomes." The macrogametes become free and are fertilized. Reduction takes place "either before copulation or during copulation, or even after the spermatozoid has already penetrated the egg." The egg nucleus, at first a collection of distinct granules, forms four chromosomes. A

division now occurs in which two chromosomes, generally smaller than the other two, are expelled from the egg. A small granule which is present at this division is interpreted as a centrosome.

This case is far from satisfactory. In the first place there is no account of any nuclear divisions prior to this "reduction" division. Consequently we do not know what the "somatic" number of the chromosomes is. We are told that the dividing nuclei "are very small and do not give good figures." It is strange that after gamete formation, when the nuclei must be considerably smaller than in the preceding divisions, Trégouboff was able to follow the reduction division "easily enough in detail, the material being particularly favourable for this study." Trégouboff's figures are unfortunately only text-figures and do not show much detail. It is to be presumed, however, that they show all the necessary points. What do they actually show? Fig. *c* is said to show "réduction chromatique dans l'œuf avant la copulation"; it actually shows, near the periphery of the egg, four round grains arranged in a square with a fifth smaller one immediately above them. Figs. *d* and *e* are said to show "réduction chromatique dans l'œuf et la copulation"; they actually show the same four granules arranged in the same way with the fifth grain occupying a similar position, while on the outside of the egg is a small black mass, presumably the microgamete. Fig. *f* is said to show "réduction chromatique après la pénétration du spermatozoïde dans l'œuf"; it actually shows once more the four round granules in square formation near the periphery of the egg, with a small black body (possibly two granules badly differentiated) lying near them; the fifth granule is absent. Fig. *g* claims to be the "syncaryon"; again there are seen the granules in "fours," the "centrosome" being absent, with two granules much smaller in size lying on the outside of the egg at the pole opposite to the "syncaryon." Fig. *i*, representing "the first mitosis in the copula," shows at one pole of a spindle (?) four granules and at the other two granules, with a dark streak joining the

two groups. Of this stage Trégouboff says: "La première division mitotique dans l'œuf fécondé donnera deux noyaux contenant chacun 4 chromosomes encore nettement distincts à ce stade, et situés aux deux pôles du sporocyste (fig. *i*); quoiqu'on ne puisse l'étudier en détail, elle paraît être du type à un long chromosome axial."

I cannot think that any evidence is here shown to prove that there is a reduction division at all. There is no trace of a division figure unless the single "centrosome" is doing duty for it. There is no sign of the "expulsion of two of the chromosomes" except that in fig. *g* the two granules on the periphery are said to be these. There is no proof that four chromosomes are present in any stage of the life-history. To establish this reduction a full series of figures showing all phases in the divisions is necessary, and until these are published it is almost impossible to determine what we are dealing with in this case. It seems to me that Trégouboff has over-estimated the ease with which he could follow the details of the reduction division. I should be inclined to class these stages with the earlier divisions—"Elles sont très petites et ne donnent pas de belles images." In the absence of any evidence showing what the somatic number of chromosomes is, I suggest that it may possibly be two, and that what Trégouboff has taken for a reduction is merely an expulsion of a portion of chromatin such as has been already described in the genus Gregarina, the four globular chromosomes arranged in square being two daughter-plates of two chromosomes each. Again I must add that, even supposing Trégouboff's account is correct, it can only apply to those gregarines whose chromosome number is even; for those with an odd chromosome number we have still to seek a method of reduction.

Other reduction processes have been discussed by some authors, but as they are of a purely speculative character, without the support of any evidence whatsoever, they seem to me to be unworthy of consideration.

It will be clear, I think, from the foregoing considerations

that the search for reduction divisions—in the proper sense of the term—in the gregarines generally has hitherto been vain. The so-called "reductions" by extrusion of chromatin granules have clearly nothing to do with the true chromosome reduction (meiosis) of other animals and plants. The attempts to discover a meiotic phase in the nuclear divisions preceding or accompanying gametogenesis have also failed. Such stages as have been found—and they are neither many nor serially complete—have been arbitrarily interpreted in such a manner as to make them fit into a general scheme of reduction and gametogenesis derived from, and therefore appropriate to, a metazoan animal. Reduction has been sought at a stage when it ought to occur according to certain preconceived notions. It has not been found, because in all probability it does not occur at this stage. The true reduction of the chromosomes occurs at a stage where it has hitherto not been sought; consequently no amount of hypothesis and argument over details has been able to surmount or explain the fundamental mistake which has been made in all the earlier attempts at interpretation.

F. The Gametes.

The forms presented by the gametes of gregarines are very varied. It would be fruitless to go over the ground that has been so carefully covered by many authors, especially by Léger and Duboscq, and the more so because the gamete nuclei—with which I am here chiefly concerned—are in many cases very much alike although the general appearances of the gametes may be so different. I shall only recall the fact that those who have studied this point seem to be of the opinion that the gametes are nearly always anisogamous. Even when isogamy has been described by one worker, more ingenious later observers have often been able to demonstrate some minute differences between the "male" and the "female" gametes. Hoffmann (1909) has pointed out that anisogamy is often more marked in the living gamete than in

the fixed and stained preparation. I confess I have not concerned myself very much with this question in Diplocystis schneideri. To me the gametes appear to be exactly alike, and I can distinguish no structural differences between the members of a pair, though it is possible that minute differences might be discoverable if more diligent search were made.

In this connection it may be recalled that the two gametes of Diplocystis, though to all appearance structurally identical, may possibly be slightly different in their functional characters. It has already been pointed out that the zygotes tend to be found towards one end of the cyst, and this would seem to indicate that the gametes formed by one of the associated individuals are more actively motile than those of the other, and migrate from one end of the cyst to seek their partners at the other end. It might therefore be justifiable to regard these two classes of gametes as "microgametes" and "macrogametes" respectively, and the organisms which form them as respectively male and female; and such a conception would, perhaps, enable us to reconcile the apparently complete structural isogamy of Diplocystis with the condition of extreme anisogamy which obtains in so many other gregarines.

One other point is also worthy of note here. In Diplocystis all the gametes formed by both individuals of an associated pair are, as we have seen, exactly alike as regards their chromosomes. Neither the "male" nor the "female" individual forms gametes distinguishable by their chromosomes into two categories. So far as can be judged at present, from the incomplete publications of others, this is also true of many other gregarines. There is no "accessory" or "sex" chromosome in these organisms, and the "unpaired" or "axial" chromosome which has been described is clearly not the homologue of the "sex chromosome" of an insect or other animal displaying a chromosomic dimorphism in its gametes. The "unpaired" chromosome of a gregarine is, indeed, unpaired: but so are all the other chromosomes which

accompany it; for the nuclei at all stages, from the first division in the gamont up to the last division before gametogenesis, contain a single set of chromosomes—a haploid and not a diploid group. This fact, if, as I believe, true of all gregarines—including those with conspicuous sexual differentiation—should be of interest to those who regard "accessory" chromosomes as "sex determinants" in the Metazoa.

6. The Sporoblast (Zygote) and its Nucleus; the True Reduction Division.

The behaviour of the nucleus in the sporoblast and its division into the eight sporozoite nuclei have never been carefully examined previously. As a rule it has been sufficient for the investigator to mention that the divisions took place—some describing a "primitive division," others ordinary mitosis. In Diplocystis schneideri for the first time the nuclear divisions within the sporocyst have been carefully examined; and, as we have seen, the results are interesting and perhaps of far-reaching importance. To recapitulate them briefly: The sporoblast is a zygote. Its nucleus is formed by the fusion of the two gamete nuclei, each containing a similar set of three chromosomes. The first nuclear division of the sporoblast is a reduction division. From the spireme, which is formed during the early prophases, six chromosomes arise. These lie on an indistinct achromatic spindle and separate into two homologous groups of three, one set of three passing to each pole. A second and a third mitotic division then take place—each presenting the same number (three) of chromosomes—giving rise, finally, to the eight sporozoite nuclei. The sporozoites thus each contain three chromosomes—a single or haploid group. This is the number present again in all the nuclear divisions—including all the divisions associated with gametogenesis—of the gamont, which is the adult organism into which the sporozoite grows.

This type of reduction division is new, and it explains in a

simple fashion the odd chromosome numbers so common among gregarines. The number found in every division excepting the first sporoblast division is the haploid number. Only once in the whole life-cycle is the diploid number found, namely, in the first division in the sporoblast. It seems probable that a careful re-examination of gregarine life-histories, paying special attention to the sporal divisions, will reveal this to be the real method of reduction in all.

Althongh Diplocystis is the only gregarine in which this type of reduction division has been found, it does not stand alone among the Sporozoa, for Dobell (Dobell and Jameson, 1915) has recorded an exactly similar phenomenon in Aggregata eberthi. Here the haploid number of chromosomes (six) occurs all through the schizogonic cycle of the parasite in the crab, and in every nuclear division in the male and female individuals in the cuttle-fish. At the first division in the zygote, however, the diploid number (twelve) occurs, consisting of two homologous groups of six differentiated chromosomes, derived from the macrogamete and microgamete nuclei. At no other stage in the life-cycle is the diploid number present. Reduction, as in Diplocystis, takes place during the first division of the zygote nucleus, the chromosome number here being halved from twelve to six. The chromosome cycle of Aggregata is thus closely comparable with that of Diplocystis, though it must be remembered that the stage in the life-history at which fertilization occurs is not the same in Coccidia and Gregarines, the zygote being a sporont in the former, a sporoblast in the latter.

Conclusions.

Of the several interesting questions which the foregoing review has touched there are only two on which I wish to lay special emphasis:

(1) The peculiar origin of the karyosome in Diplocystis schneideri, in which a "micronucleus" makes its way inside a nucleolus, giving rise to a "karyosome" composed of

two clearly differentiated portions, taken in conjunction with the fact that in many other Gregarines the karyosome is similarly constructed, opens, I think, a very important question. The entire gregarine "nucleus" is clearly not exactly comparable with the nucleus of a metazoan cell. It is probably a much more complex organ, comparable with a nucleus within a nucleus. Such evidence as I have been able to bring forward is insufficient to exhaust this question, which is undoubtedly one that would repay further inquiry.

(2) Heretofore, reduction in gregarines has been sought for in the two nuclear divisions immediately preceding gamete formation. I have tried to show that in none of the so-called "reduction divisions" which have been described has a true reduction been demonstrated. In Diplocystis schneideri the reduction division has been found to be the first division in the sporoblast, and this is probably the stage at which it occurs in other forms. This mode of reduction offers a simple explanation of the odd chromosome number which is so common in gregarines; it is the haploid number, which is present in every nucleus in the whole life-cycle except the zygote nucleus.

Finally I would like to draw attention to the fact that both of the above important points emphasize the difference between the Protozoa and the Metazoa. In the present case, especially as regards reduction, a somewhat slavish following of metazoan embryology has, indeed, already obscured the truth and led into serious error. Dobell (1911) has shown the futility of interpreting the Protozoa in terms of the metazoan cell, and yet it still goes on. Clarity of thinking will not come in Protozoology until the Protozoa are fully recognized as non-cellular organisms, comparable with whole metazoan individuals rather than with their single component cells.

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EXPLANATION OF PLATES 12 TO 15.

Illustrating Mr. A. Pringle Jameson's paper on "The Chromosome Cycle of Gregarines, with Special Reference to Diplocystis schneideri Kunstler."

[The figures were all drawn with the aid of a camera lucida. Excepting for figs. 1, 25, 26 and 27 a Leitz 2 mm. apochromatic lens with compensating oculars was used. Unless stated otherwise the figures are from sections stained with Dobell's hæmatein stain and light green.]

PLATE 12.

Fig. 1.—A Diplocystis "pair" from the body-cavity of a cockroach, showing in each a large nucleus with several karyosomes. Whole mount. Borax carmine. × 65.

Fig. 2.—Spore showing sporozoites coiled within. Smear from ripe cyst. Acid carmine. × 1600.

Fig. 3.—Spore showing line of dehiscence. Fresh. × 1600.

Fig. 4.—Empty sporocyst showing triangular opening and sporozoite lying beside it. Smear from gut of cockroach fed on spores. × 1600.

Fig. 5.—Sporozoite showing nucleus. It has become rather flattened in the preparation. Smear. × 2500.

Fig. 6.—Spore commencing to dehisce. × 2500.

Fig. 7.—Sporozoite in gut-wall. Smear. × 1600.

Fig. 8.—Sporozoite in gut-wall. Growth has commenced. The oval netted bodies are the nuclei of the cells. × 1600.

Fig. 9.—Sporozoite in gut-wall. Later stage showing amœboid appearance. × 1600.